bio-protocol Preprint

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Coimmunoprecipitation and immunoblot

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An abbreviated version of this protocol was published in Science Immunology in May 2022

Rgs16 promotes antitumor CD8+ T cell exhaustion

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Detailed protocol

Co-immunoprecipitation and immunoblot

1. Harvest T cells, spin down 2000 rpm, 1min;
2. Cells were lysed with RIPA buffer (Santa Cruz Biotechnology), on ice, 10 min.
3. Spin down, and collect the lysis buffer in the supernatant.
4. Incubate the lysates with Protein A Dynabeads pre-coated with the indicated antibodies 4'C, overnight.
5. Spin down and collect the Protein A Dynabeads. Protein A Dynabeads are supposed to be bound with the protein complex now.
6. Wash Protein A Dynabeads 3 times.
7. Boil Protein A Dynabeads.
8. Spin down again to collect the supernatant.
9. Load the supernatant to SDS- PAGE.
10. Run SDS-PAGE (120 V) for about 1.5 hours.
11. Transferred proteins from the gel onto a PVDF membrane. 100 V for 1 hour.
12. The PVDF membrane was blocked with 1% BSA in PBS supplemented with Tween-20 (PBST) (1 hour, room temperature).
13. The PVDF membrane was then incubated overnight at 4°C with primary antibodies.
14. The PVDF membrane was washed (3 times, 10 minutes each time) with PBST.
15. The PVDF membrane was incubated with HRP-conjugated secondary antibodies (1 hour, room temperature). We used regular (cat# 406401, BioLegend) or conformation specific (cat# 5127S, Cell Signaling Technology) HRP-conjugated secondary antibodies. Notably, the regular secondary antibody produced a non-specific 100–130 kDa band in the lane with IgG (cat# I5006, Sigma-Aldrich) mock immunoprecipitation, but this band was much weaker when the conformation specific secondary antibody was used.
16. The PVDF membrane was washed (3 times, 10 minutes each time) with PBST.
17. The membrane was developed with the ECL method. The data were collected with a Fusion imaging system (FX6 Edge, Vilber). Protein markers were merged within the immunoblot images.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Cui, G. (2022). Coimmunoprecipitation and immunoblot. Bio-protocol Preprint. bio-protocol.org/prep1929.
2. Weisshaar, N., Wu, J., Ming, Y., Madi, A., Hotz-Wagenblatt, A., Ma, S., Mieg, A., Hering, M., Zettl, F., Mohr, K., Schlimbach, T., Ten Bosch, N., Hertel, F., Müller, L., Byren, H., Wang, M., Borgers, H., Munz, M., Schmitt, L., van der Hoeven, F., Kloz, U., Carretero, R., Schleußner, N., Jackstadt, R., Hofmann, I. and Cui, G.(2022). Rgs16 promotes antitumor CD8+ T cell exhaustion. Science Immunology 7(71). DOI: 10.1126/sciimmunol.abh1873